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Current Opinion in Plant Biology

Broad compatibility in fungal root symbioses

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Plants associate with a wide range of beneficial fungi in their roots which facilitate plant mineral nutrient uptake in exchange for carbohydrates and other organic metabolites. These associations play a key role in shaping terrestrial ecosystems and are widely believed to have promoted the evolution of land plants. To establish compatibility with their host, root-associated fungi have evolved diverse colonization strategies with distinct morphological, functional and genomic specializations as well as different degrees of interdependence. They include obligate biotrophic arbuscular mycorrhizal (AM), and facultative biotrophic ectomycorrhizal (ECM) interactions but are not restricted to these well-characterized symbioses. There is growing evidence that root endophytic associations, which due to their inconspicuous nature have been often overlooked, can be of mutualistic nature and represent important players in natural and managed environments. Recent research into the biology and genomics of root associations revealed fascinating insight into the phenotypic and trophic plasticity of these fungi and underlined genomic traits associated with biotrophy and saprotrophy. In this review we will consider the commonalities and differences of AM and ECM associations and contrast them with root endophytes.

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Introduction

Beneficial root-associated fungi perform vital functions in host mineral nutrient uptake, carbon (C) cycling, plant growth promotion and/or increased resistance against plant pathogens that are fundamental to sustainable plant productivity. This is achieved by the establishment of an intimate interaction between the host cells and the fungal hyphae that can be more or less extensive and limited to the epidermis or include the cortex layers. These multifaceted fungal symbioses comprise a full spectrum of variation forming a continuum of interactions with highly distinct anatomical features and separate evolutionary histories [1–4]. The obligate biotrophic arbuscular mycorrhizal (AM) fungi belong to the Glomeromycota phylum, one of the oldest fungal lineages, and form the most widespread and common root–fungus associations. AM fungi have evolved an efficient means of acquiring inorganic nutrients from soil to supply plants, but cannot grow apart from their hosts [3,5,6]. Therefore, they are thought to have none or very little saprotrophic capability [7••,8••]. Ectomycorrhizal (ECM) fungi have arisen independently several times from saprotrophic ancestors and can be found in the phyla Ascomycota and Basidiomycota [9,10]. These fungi are important in forest ecosystems and, although they are capable to colonize the surface of non-host roots without penetrating them, intercellular growth is restricted to specific plant families, mostly trees [6]. These dual soil–plant inhabitants are efficient at deriving nutrients saprotrophically from soil organic matter, where they live transitorily, and biotrophically from plants, during mutualistic interactions. Thus, they display a strong adaptation to life within hosts but have maintained saprotrophic characters [11••,12••]. Depending on environmental conditions and host partners, ECM fungi can additionally be involved in parasitism where fungal infections may lead to the production of severe necrosis in the root cortices [13–16], indicating potential for mutualism and pathogenicity in this group of fungi. A different class of root associations is represented by the non-mycorrhizal endophytes. This group of fungi can be of beneficial nature and while the underpinning mechanisms are largely unknown, plant benefits range from growth promotion to increased resistance to biotic and abiotic stresses [17]. By definition root endophytes do not form an interface of specialized hyphae and are thought to colonize the host without efficient means for nutrient transfer towards the host [18]. Yet recent evidence shows that these fungi can form extensive biotrophic interfaces with plant cells, during which fungal hyphae are encased by the host plasma membrane [19,20•]. Indeed in several endophytic interactions nutrient transfer between the two partners was reported, but the means of transfer at the biotrophic interface is still unclear [21–23]. These fungi are widespread root inhabitants closely related to, but not restricted to ECM, orchid mycorrhiza (OM) and ericoid fungi, and also insect-parasitic fungi can act as beneficial plant endophytes delivering the roots with insect-derived nitrogen (N) [21,24]. Some mycoparasitic fungi feeding on other fungi can also be classified as beneficial root

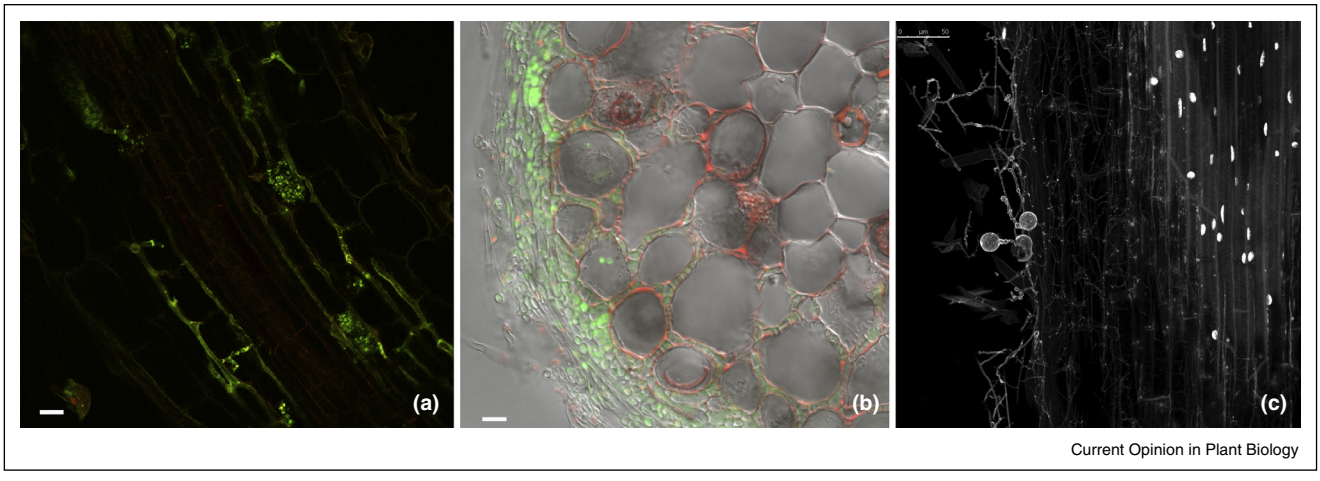
endophytes. These fungi are widely used in agriculture as biocontrol agents and whereas the mycoparasitism represents the ancestral life style they have acquired the ability to grow between cortical cells of their plant hosts [25•,26]. Like the AM fungi, root endophytes have a wide host range and can be found associated with the so-called non-mycorrhizal (NM) plants where they are able to establish biotrophy [19,20•,27]. Endophytic colonization of NM plants by AM fungi has also been reported, but it is considered to be functionally less significant as no arbuscules are formed in these hosts and hyphae typically occur in moribund cells with no plant growth promotion [18,28].

Commonalities and differences in AM, ECM and endophytic fungi, while sometimes difficult to grasp, are important to understand the impact of individual symbiotic interactions in the ecosystem and might be reflected in their genomic and transcriptomic traits. The recent release of the genomes of the AM fungus *Rhizophagus irregularis* (formerly known as *Glomus intraradices*) [7••,8••], the ECM fungi, *Laccaria bicolor* [11••] and *Tuber melanosporum* [12••], and the root endophyte *Piriformospora indica* [29••] provides unprecedented insights into how these beneficial root symbionts penetrate and establish within their hosts and to which extent their lifestyles are encoded in their genomes. This review describes current advances in understanding the components of root endophytic lifestyles from biological and comparative genomic analyses.

Biology of the symbiotic interface

The obligate biotrophic AM fungus *R. irregularis* (Glomeromycota, Glomerales) forms highly branched, tree-shaped structures, the arbuscules, inside living cortical cells, preferentially in the inner layers (Figures 1a, 2a). This extensive interface was shown to be the site of symbiotic nutrient transfer where phosphate and N are actively transferred to the plant in exchange for simple carbohydrates [3,5,30,31]. These fascinating fungal structures are associated with dramatic reprogramming of the host cell to accommodate intracellular hyphae which start even before actual penetration, resulting in the so-called pre-penetration apparatus [32]. Host cell rearrangement includes remodeling of actin filaments and microtubules, movement of the host nucleus to the center of the cell and site of fungal penetration, and deformation of the vacuole with proliferation of plastids and mitochondria. Intense re-organization of host cell architecture and physiology seems to be characteristic of obligate biotrophy and can be paralleled in mutualists and pathogens (e.g. powdery mildew fungi) [33], reflecting a continued coevolution with the hosts that led to the development of fungal and plant tools efficiently tailored to each other. Successful colonization and beneficial outcome by AM fungi is indeed dependent on the presence of a common symbiosis signaling pathway (SYM pathway) in the hosts [34,35]. This pathway is functionally conserved in several plant families and has homologs in bryophytes and green algae of the order Charales, suggesting the remote possibility of symbiotic associations in green algae [36].

Figure 1



(a) Section of paraffin-embedded root of *M. truncatula* inoculated with *R. irregularis* after staining with fluorescein isothiocyanate conjugate-wheat germ agglutinin, WGA-FITC. Scale bar, 10 μm. Photo kindly provided by Raffaella Balestrini and Paola Bonfante. **(b)** Laser-scanning confocal microscopy image of a transverse section of 12-week-old *L. bicolor–Populus trichocarpa* ectomycorrhiza root tip. Green signal corresponds to indirect immunolocalization of *L. bicolor* MiSSP8 protein (unpublished data) and plant root cells are counterstained with propidium iodide in red. Scale bar, 10 μm. Photo kindly provided by Claire Veneault-Fourrey. **(c)** Maximum projection of a barley root colonized by *P. indica* at 30 days post inoculation. Broad extraradical hyphae are visible at the boundary of the epidermis, whereas thin secondary hyphae are filling the cortical cells. Host nuclei are absent in the cortex cells, while the cylinder is undamaged and preserves intact nuclei. Scale bar, 50 μm.

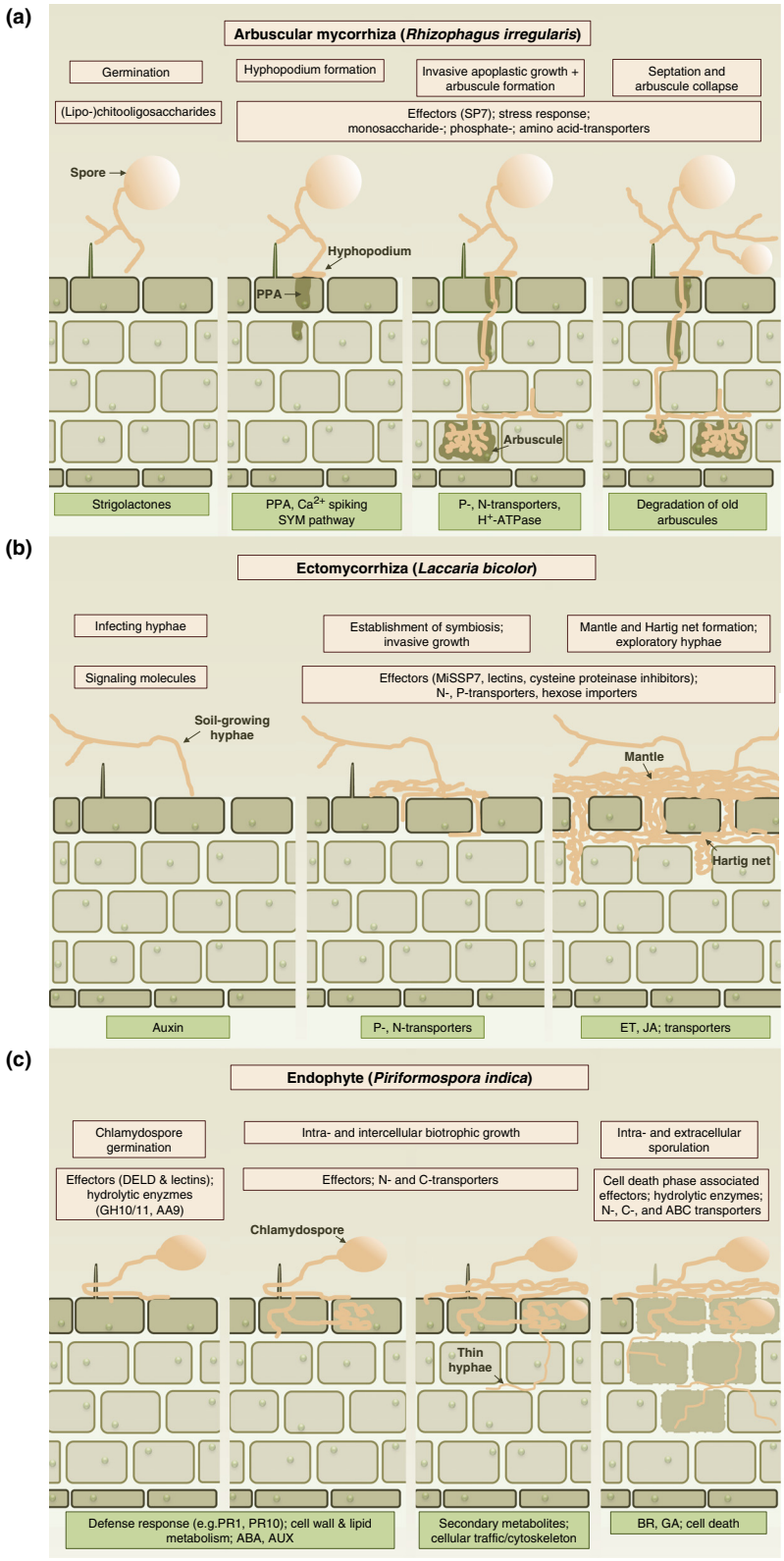
ECM associations with plants by the fungi *L. bicolor* (Basidiomycota, Agaricales) and *T. melanosporum* (Ascomycota, Pezizales) are characterized by the production of a sheath of organized hyphae, which encloses the fine lateral roots, and by the Hartig net formed by hyphae penetrating the anticlinal space of adjacent rhizodermis cells and the outer layers of the root cortex [37] (Figures 1b, 2b). Intercellular and extraradical hyphae are thought to have different functions. The Hartig net represents the biotrophic interface between host cells and fungal hyphae where communication and nutrient exchange between the two partners occur. The fungal sheath serves as an intermediate storage compartment for nutrients originating from the host via the Hartig net and from the soil-growing hyphae [2,6,38,39]. It was recently suggested that ECM fungi do not take up sucrose but glucose secreted by the plant via mycorrhizal-induced hexose facilitators [2]. In return, ECM fungi supply the plant with phosphate [40] and eventually N, although the possible N-flow from the plant to the fungus via uptake of plant-derived amino acids and proteins from the apoplast has also been discussed [2,6]. Unlike AM fungi, ECM fungi are not strictly dependent on the host, but in natural forest ecosystems, where major nutrients are fixed in complex organic matter, ECM interactions help overcoming nutritional limitations faced by both partners, thus a substantial degree of coevolution and specialization in this group of fungi is expected [9].

The root endophyte *P. indica* (Basidiomycota, Sebacinales) establishes an intermediate form of root association with characteristics of both ectomycorrhizae and endomycorrhizae (Figures 1c, 2c). During colonization with many different plant families, *P. indica* forms an external loose network of hyphae. Additionally, fungal hyphae intercellularly and intracellularly colonize the root epidermal layer and, depending on the host, the outer cortex cells [19,41–43]. *Piriformospora indica* was reported to be able to undergo beneficial relationships with a broad range of experimental host species, including the dicotyledonous NM plant *Arabidopsis thaliana* [44] and the monocotyledonous barley [41] and to deliver phosphate to the plant [22], although an induction of mycorrhizal specific plant phosphate transporters could not be observed [45]. Beside its capability to colonize roots intracellularly, this symbiont is able to gain organic nutrients by degrading dead root material saprotrophically [29••]. The dual lifestyle of *P. indica* is also evident during mutualistic fungal development in the roots of barley and *Arabidopsis* where it displays a biphasic colonization strategy. Upon penetration of the root, *P. indica* establishes a biotrophic interaction where hyphae are enveloped by the host plasma membrane in viable cells. Later, *P. indica* hyphae are found more often in dead or dying host cells where they secrete a large variety of hydrolytic enzymes that degrade plant cell walls and proteins, especially in the root cortex of barley [20•,29••,41,46]. The expression of extracellular proteases and metalloproteases in *P. indica* could represent an alternative nutritional strategy where demands for C and N may be satisfied by protein degradation during the switch from biotrophy to the cell-death associated phase [4]. Although a defined switch to necrotrophy with massive cell death and tissue maceration is missing and instead beneficial effects for the hosts are present, this strategy of colonizing plants resembles that of hemibiotrophic fungi, straddling the divide of saprotrophy, necrotrophy and mutualism [47]. The maintenance or enforcement of saprotrophic characters in this fungus together with the implementation of biotrophic traits have possibly led to the ability to colonize a large number of unrelated hosts, making this fungus a classical generalist [4]. Whether beneficial outcome of the interaction with a broad range of plants is based on general mechanisms and signaling pathways common to many plant families, as described for AM fungi, remains an open question.

Host-dependent colonization strategies in root symbioses

To establish and maintain a compatible interaction with diverse hosts, mutualistic and pathogenic fungi must evolve highly adaptive capacities to cope with a plethora of different host-specific signals, resulting in the expansion and diversification of the fungal toolkit and its expression in a host-dependent manner. Alternative lifestyles and colonization strategies may thus be a consequence of this adaptation to highly variable environments. Recently it was shown, by cytological studies and global investigations of *P. indica* transcriptional responses to colonization of barley and *Arabidopsis* at different symbiotic stages, that broad compatibility is associated with host-dependent colonization strategies and with host-specifically-induced effector candidates [20•]. In *Arabidopsis*, *P. indica* establishes and maintains predominant biotrophic nutrition within living epidermal cells with production of bulbous hyphae, while in barley the symbiont undergoes a nutritional switch to saprotrophy that is associated with the production of thinner hyphae in cortex cells [20•]. Consistent with the occurrence of N limitation at the onset of saprotrophy in barley, the concentrations of free amino acids (aa) in the older root zone of barley are remarkably lower compared to the early stage, irrespective of *P. indica* colonization [20•]. In *Arabidopsis*, colonization by *P. indica* significantly increases the level of free aa at the infection zone. The altered organic N allocation is mainly due to changes in asparagine, glutamine and threonine which might represent a ready source of organic N during biotrophy as described in other biotrophic interactions [48]. These results contribute to the finding that different host metabolic environments affect the colonization strategies in root endophytes. Extensive host metabolic reprogramming occurs also during *L. bicolor* colonization [49]. This reprogramming is host-dependent, indicating that in

Figure 2



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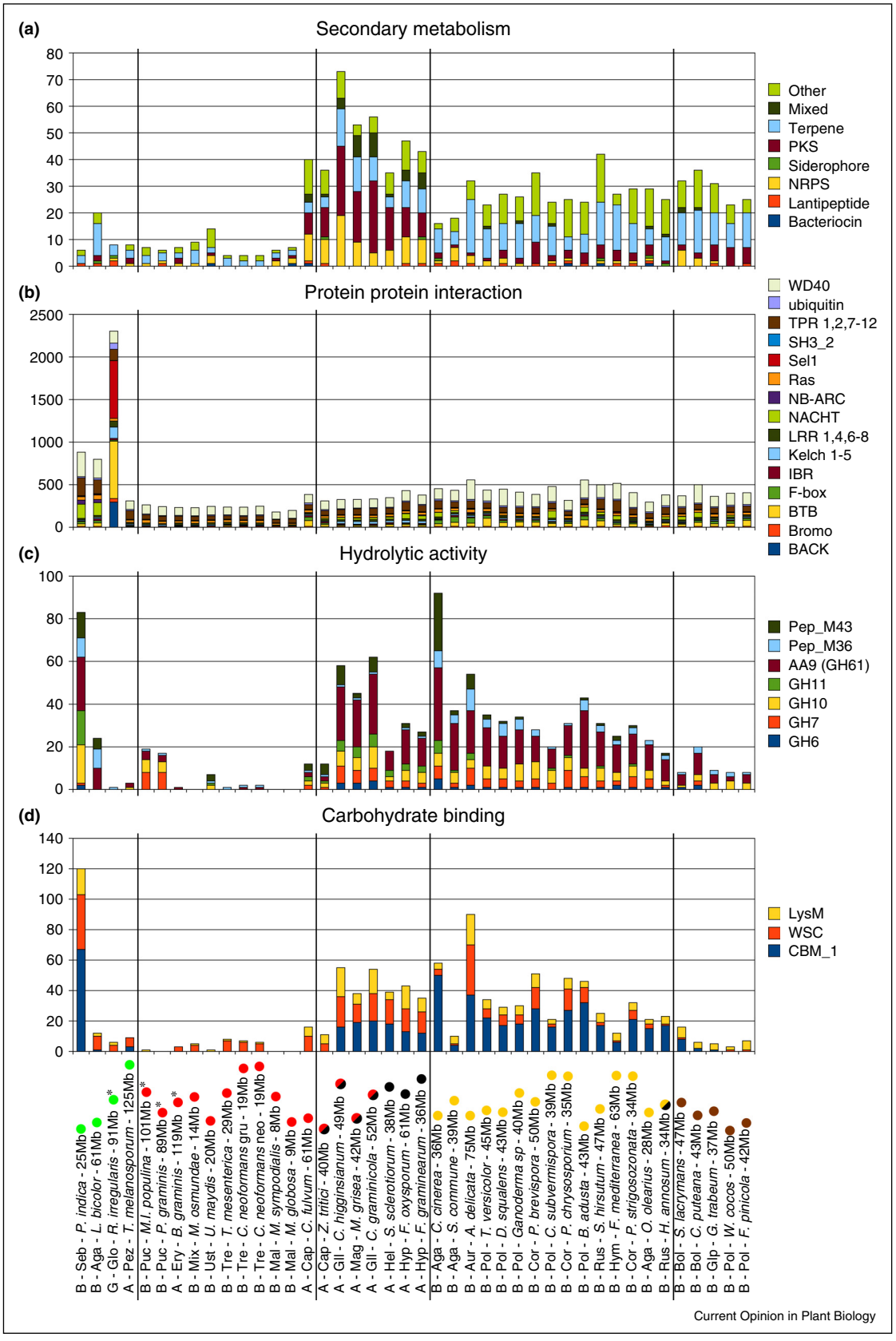
ECM fungi the metabolic responsiveness of plant roots is a determinant factor in the interaction. Host-specific colonization strategies with different morphological patterns have long been known in AM fungi [50–52], suggesting that this may represent a common feature in broad compatibility in root symbioses. Knowledge of the molecular and genetic mechanisms regulating AM colonization strategies in different hosts is still limited and it is unclear whether the establishment of different fungal structures in different hosts is driven by host-related metabolic cues. The release of the first AM fungal genome [7••,8••] will conspicuously speed up our understanding of the fungal partner in this symbiosis.

Is fungal lifestyle reflected in the genomic traits of root symbionts?

Fungal lifestyles and the level of specialization to the host are expected to influence the evolution of genomic traits and of effector proteins involved in the establishment of compatibility. Root-associated fungi show a great variability in colonization and nutritional strategies and although the examples we discuss in this review are all defined as biotrophic mutualistic associations where both the fungus and the plant benefit from each other, their lifestyles range from obligate biotrophy to hemibiotrophy with more or less marked saprotrophic characters. Additionally, fungal colonization and nutritional strategies may vary depending on the host, thus it becomes evident that standard categories cannot be applied to define root symbionts. Detailed analyses of the saprotrophic capabilities and colonization strategies of these fungi in different hosts must be carefully performed and definitions applied on a case-by-case basis. Genomics and transcriptomics together with cytological and biochemical studies provide valuable clues to understanding the potentiality of these fungi. In particular comparative genome analyses recently succeeded in shedding some light on the possible common and specific genetic features in such a heterogeneous set of root–fungus associations. One common genomic feature is represented by the low number of genes involved in secondary metabolism, which are overrepresented in necrotrophic and saprotrophic fungi [7••,11••,12••,29••]. This feature is reflected in the genomes of obligate and non-obligate biotrophic pathogens (Figure 3a), indicating convergent adaptation to a life inside living host cells [53•,54•]. In the genome of *R. irregularis* a dramatic expansion of genes encoding proteins containing domains whose functions are related to signaling transduction via phosphorylation (e.g. tyrosine kinases) and regulation of gene expression and protein levels (ubiquitin, BACK-domains, Kelch-domains, LRR-domains, Sel1-domains, Bromo-domains and BTB/POZ-domains) is present (Figure 3b). These functional domains are involved in protein–protein interactions with multiple cellular roles, such as recruitment to E3 ligase complexes and in organization of the cytoskeleton via interaction with actin and intermediate filaments [55]. This is not surprising considering the pivotal role of the perception of environmental signals for association with plants and the dramatic morphological changes associated with establishment of biotrophy in this fungus. Expansion for gene families containing domains involved in protein–protein and protein–DNA interactions was also observed for *L. bicolor* and *P. indica* (e.g. WD40-domains, F-box-domains, Bromo-domains, TPR-domains, NB-ARC-domains, NACHT-domains, IBR-domains and SH3_2-domains) and to a lesser extent also for *T. melanosporum* (Figure 3b), suggesting that these could represent a common genomic feature in root associations where the fungus undergoes complex changes in anatomical structures (coils, arbuscules, multilobed hyphae and thin hyphae), lifestyle (between soil-growing hyphae and biotrophic hyphae inside the host) and interaction partners (soil-living microbes and plant hosts). Various genomic trends have been discussed as relevant for a symbiotic lifestyle, such as larger genomes [47,53•] (Figure 4), abundance of transposable elements, expansion of multigene families [56,57], presence of a large repertoire of *in planta* induced small secreted proteins (SSPs < 300 aa) [4,57] or the absence/reduction of genes

(FIgure 2 Legend) (a) Germination of AM spores and hyphal branching is stimulated by strigolactones exuded by the roots. The fungus produces signaling molecules such as lipochitooligosaccharides, which induce calcium spiking, lateral root formation and changes in C-metabolism [59–61]. After establishment of the hyphopodium on the root surface, the pre-penetration apparatus (PPA) is built as a transvacuolar structure guiding microbial invasion. Several SYM genes were identified to be required for establishment of symbiosis with AM fungi as well as N-fixing rhizobia [34]. Effectors are thought to suppress initial defense response of the plant as it was demonstrated for the effector SP7 which interacts with the host transcription factor ERF19 (ethylene response factor 19) [62]. Arbuscules are formed inside living cells where nutrients like monosaccharides and phosphate are exchanged under the control of both partners [56,63]. The symbiotic partners form a long-lasting interaction, while individual arbuscules collapse and are degraded in viable host cells [3]. **(b)** After first contact with the roots of mycorrhizal plants, ECM fungi produce a mantle at the root tips and successively the Hartig net. Colonization by the fungus triggers accumulation of auxin at root tips and lateral root formation in mycorrhizal as well as non-mycorrhizal plants [64]. To establish a mutualistic symbiosis, putative effectors like lectins, proteinase inhibitors and small proteins (SSPs) are secreted [11••,39]. Some of these SSPs are translocated into the host cell as demonstrated for MiSSP7 [65]. N and phosphate are supplied to the root by the Hartig net and C is taken up in the form of monosaccharides. At late stages of colonization, ethylene (ET) and jasmonate (JA) responsive genes are induced in the root to limit fungal colonization [66]. **(c)** Germinated chlamydospores or infecting hyphae of the mutualistic endophyte *P. indica* attach to and penetrate the rhizodermis cells triggering initial defense responses and alterations in abscisic acid (ABA) and auxin (AUX) metabolism [67–69]. Subsequently, biotrophic hyphae grow inside living cells with suppression of host defense responses and expression of lectins and small secreted proteins like DELD effectors [19,29••]. During intracellular and intercellular colonization of the cortex, fungal N and carbohydrate transporter genes are induced [4,20,29••]. During the cell-death associated phase, fungal hydrolytic enzymes and ABC transporters are activated and alterations in brassinolide and gibberellic acid metabolism are observed in the roots [20,29••].

Figure 3


(a)
Secondary metabolism
Other
Mixed
Terpene
PKS
Siderophore
NRPS
Lantipeptide
Bacteriocin
(b)
Protein protein interaction
WD40
ubiquitin
TPR 1,2,7-12
SH3_2
Sel1
Ras
NB-ARC
NACHT
LRR 1,4,6-8
Kelch 1-5
IBR
F-box
BTB
Bromo
BACK
(c)
Hydrolytic activity
Pep_M43
Pep_M36
AA9 (GH61)
GH11
GH10
GH7
GH6
(d)
Carbohydrate binding
LysM
WSC
CBM_1
B - Seb - P. indica - 25Mb
B - Aga - L. bicolor - 61Mb
G - Glo - R. irregularis - 91Mb
A - Pez - T. melanosporum - 125Mb
B - Puc - M.l. populina - 101Mb
B - Puc - P. graminis - 89Mb
A - Ery - B. graminis - 119Mb
B - Mix - M. osmundae - 14Mb
B - Ust - U. maydis - 20Mb
B - Tre - T. mesenterica - 29Mb
B - Tre - C. neoformans gru - 19Mb
B - Tre - C. neoformans neo - 19Mb
B - Mal - M. sympodialis - 8Mb
B - Mal - M. globosa - 9Mb
A - Cap - C. fulvum - 61Mb
A - Cap - Z. tritici - 40Mb
A - Gll - C. higginsianum - 49Mb
A - Mag - M. grisea - 42Mb
A - Gll - C. graminicola - 52Mb
A - Hel - S. sclerotiorum - 38Mb
A - Hyp - F. oxysporum - 61Mb
A - Hyp - F. graminearum - 36Mb
B - Aga - C. cinerea - 36Mb
B - Aga - S. commune - 39Mb
B - Aur - A. delicata - 75Mb
B - Pol - T. versicolor - 45Mb
B - Pol - D. squalens - 43Mb
B - Pol - Ganoderma sp - 40Mb
B - Cor - P. brevispora - 50Mb
B - Pol - C. subvermispora - 39Mb
B - Cor - P. chrysosporium - 35Mb
B - Pol - B. adusta - 43Mb
B - Rus - S. hirsutum - 47Mb
B - Hym - F. mediterranea - 63Mb
B - Cor - P. strigosozonata - 34Mb
B - Aga - O. olearius - 28Mb
B - Rus - H. annosum - 34Mb
B - Bol - S. lacrymans - 47Mb
B - Bol - C. puteana - 43Mb
B - Glp - G. trabeum - 37Mb
B - Pol - W. cocos - 50Mb
B - Pol - F. pinicola - 42Mb
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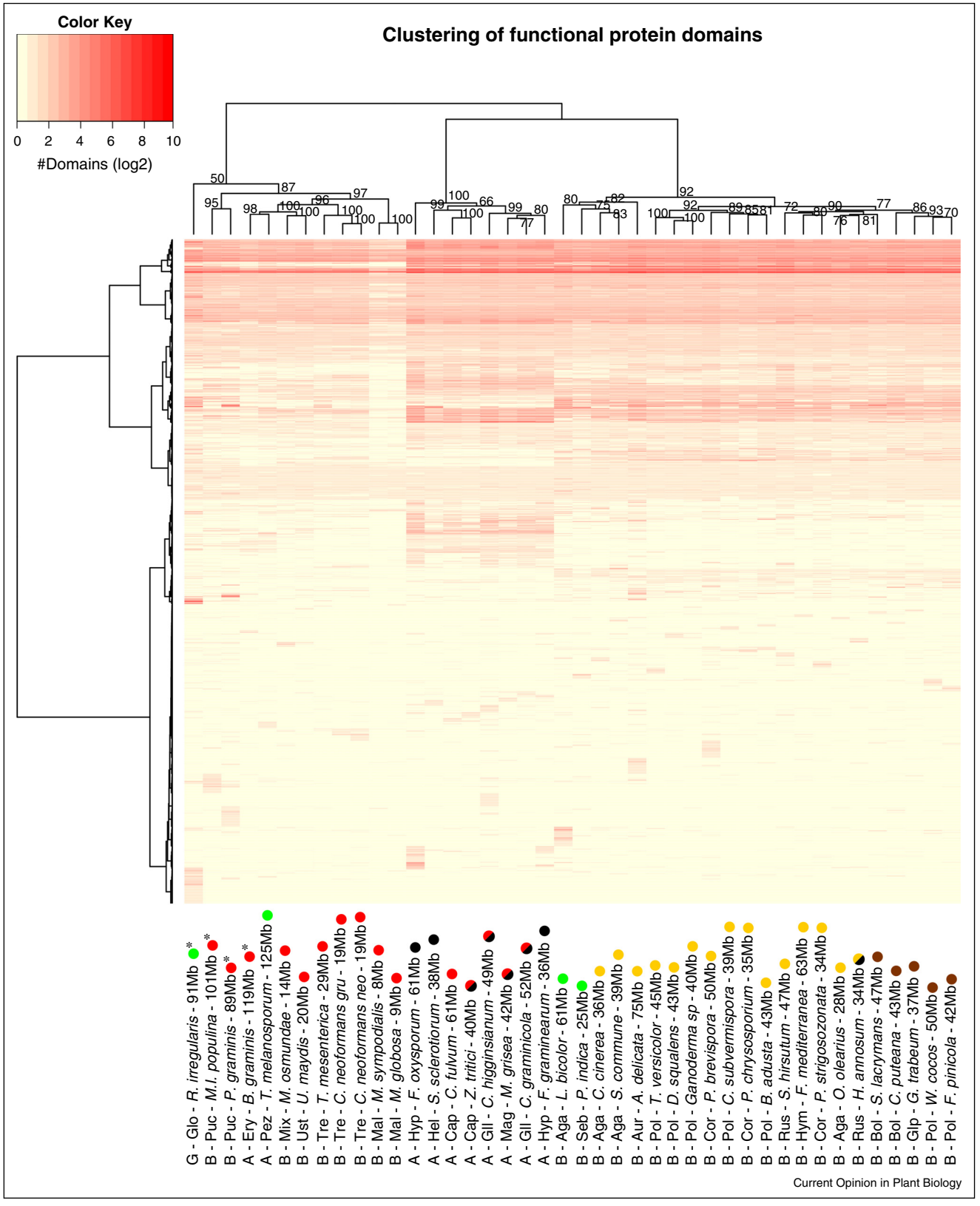
involved in N uptake, plant cell wall degradation [29••,53•] and secreted invertases [7••,11••,12••,56,57]. With each released genome it became evident that none of these traits is mandatory for symbiosis [7••,9,11••,12••,29••,57]. Also the expansion expected for genes encoding carbohydrate transporters was not detected in the genomes of mutualistic symbionts. Carbohydrate transporters are actually underrepresented compared to necrotrophic fungi, suggesting that uptake of different carbohydrates is more important during necrotrophy, when the pathogen uses dead and dying host cells as a nutrient source to support rapid colonization and sporulation [58•]. An interesting feature of the *R. irregularis* genome is the small number of predicted secreted proteins in comparison to other pathogenic and symbiotic fungal genomes [8••]. The secretome of *R. irregularis* has been streamlined through the loss of genes involved in saprotrophic growth with few small secreted proteins that are induced *in planta* [7••,8••]. With respect to the effectors of mutualistic fungi, one of the challenges will be to determine their role in the establishment of compatibility with a wide range of hosts.

The number of published genomes for symbiotic fungi is still quite small. Nevertheless, data are valuable to infer lifestyle complexity, showing that root-associated fungi possess species-specific saprotrophic characteristics (Figure 3c,d). This is confirmed by clustering analysis of functional domains (Figure 4), underscoring the polyphyletic origins of these symbioses and their diverse nutritional strategies. Fungi with an obligate or predominant biotrophic lifestyle cluster well together, demonstrating that this habit is well reflected in their genomes. This is also true for necrotrophs and hemibiotrophs. A clear separation can also be found between white and brown rot saprotrophs, independently from their phylogenetic positions, suggesting a strong relationship between lifestyle and expansion/contraction of functional domains in the genomes of these fungi. The dual lifestyle of *P. indica* is also well reflected in its genome. This is shown, among others, by the presence of genes involved in plant cell wall degradation (e.g. Glyco hydro GH6, GH7, GH10, GH11 and AA9 formerly known as GH61) and protein hydrolysis (e.g. Metallopeptidases M36 and M43) which are strongly reduced or absent in obligate biotrophs, but well represented in the genomes of white rot fungi (Figure 3c). Both *T. melanosporum* and *L. bicolor* still have a residual ability to degrade plant cell walls but the hydrolytic gene classes differ in these two ECM fungi [57]. The diverging enzymatic arsenal and the induction of these genes in symbiotic tissues in *T. melanosporum* and *P. indica* but not in *L. bicolor* suggests a different colonization strategy where *T. melanosporum* and *P. indica* may act more aggressively towards their hosts [20•,57]. Indeed in both fungi the degradation of plant cell walls during symbiotic interaction and induction of genes involved in lipid and protein degradation was observed [12••,29••]. Global transcriptional responses associated with colonization of barley and *Arabidopsis* by *P. indica* showed that members of the AA9, GH10 and GH11 families were induced in barley but to a lesser extent in *Arabidopsis*. It may well be that host specialization influenced the amount and type of genes encoding hydrolytic enzymes in the genomes of symbiotic fungi. In support of this idea is the fact that genes encoding AA9 and GH10 are overrepresented in the genome of the hemibiotrophic pathogen *Colletotrichum graminicola* which primarily infects maize, compared to the genome of the closely related *C. higginsianum*, a pathogen of several members of Brassicacea reflecting the different cell wall compositions of monocots and dicots [58•].

(Figure 3 Legend) Comparison of proteins containing different domains involved in secondary metabolite biosynthesis, hydrolytic activity, protein–protein interaction and carbohydrate binding from 42 fungal species of the Basidiomycota, Ascomycota and Glomeromycota phyla. Shown is a selection of gene families which proved to be either expanded or contracted in the genomes of *P. indica*, *L. bicolor*, or *R. irregularis* based on comparative analyses. Proteins of publically available genomes were annotated using the Pfam database version 27 [70]. The numbers of proteins containing one of the selected domains are shown in the *y*-axis. Fungi are grouped based on their predominant lifestyle into symbionts (green dots), biotrophic plant and animal pathogens (red dots), hemibiotrophic (red/black dots) and necrotrophic plant pathogens (black dots), white rot saprotrophs (yellow dots) and brown rot fungi (brown dots). Proteins involved in secondary metabolite biosynthesis and hydrolyses are expanded in the genomes of necrotrophs, hemibiotrophs and saprotrophs. An exception is the biotrophic tomato pathogen *C. fulvum* which displays a large arsenal of carbohydrate-degrading enzymes but many of these genes are not expressed *in planta* or are pseudogenized [71•]. Gene families encoding proteins involved in signaling are expanded in symbionts whereas expansion for gene families encoding lectins seems to be a specific feature of the genus Sebacinales (e.g. *P. indica*). Asterisks indicate obligate biotrophy. **(a)** Number of proteins and protein clusters predicted to be involved in antibiotic and secondary metabolite production. The prediction was performed using the stand-alone version of antiSMASH v.2 [72] with standard settings. **(b)** Number of proteins containing one of the following domains involved in protein–protein interaction and regulation: WD domain, G-beta repeat (WD40, PF00400); ubiquitin family (ubiquitin, PF00240); tetratricopeptide repeat class 1, 2 and 7–12 (TPR_1, PF00515; TPR_2, PF07719; TPR_7, PF13176; TPR_8, PF13181; TPR_9, PF13371; TPR_10, PF13374; TPR_11, PF13414; TPR_12, PF13424); variant SH3 domain (SH3_2, PF07653); Sel1 repeat (Sel1, PF08238); Ras family (Ras, PF00071); NB-ARC domain (NB-ARC, PF00931); NACHT domain (NACHT, PF05729); leucine rich repeat class 1, 4 and 6–8 (LRR_1, PF00560; LRR_4, PF12799; LRR_6, PF13516; LRR_7, PF13504; LRR_8, PF13855); kelch motif class 1–5 (Kelch_1, PF01344; Kelch_2, PF07646; Kelch_3, PF13415; Kelch_4, PF13418; Kelch_5, PF13854); IBR domain (IBR, PF01485); F-box domain (F-box, PF00646); BTB/POZ domain (BTB, PF00651); bromo (Bromodomain, PF00439); BTB and c-terminal Kelch (BACK, PF07707). **(c)** Number of proteins containing one of the following enzymatic domains: pregnancy-associated plasma protein-A (Pep_M43, PF05572); fungalysin metallopeptidase (Pep_M36, PF02128); copper-dependent lytic polysaccharide monooxygenases (AA9, formerly GH61, PF03443); glycoside hydrolase family 11 (GH11, PF00457); glycoside hydrolase family 10 (GH10, PF00331); glycoside hydrolase family 7 (GH7, PF00840); glycoside hydrolase family 6 (GH6, PF01341). D: Number of proteins containing one of the following carbohydrate-binding domains: lysin motif domain (LysM, PF01476); cell wall integrity and stress response component domain, (WSC, PF01822); and carbohydrate-binding module 1 (CBM_1, PF00734).

Figure 4



Clustering analysis of functional protein domains results in the separation of fungal groups based on their lifestyles and phylogenetic position. Proteins of publically available fungal genomes were downloaded from the MycoCosm portal of the JGI [73] and annotated using the Pfam database V.27 [70].

Conclusions

What do we learn from comparative genomics and transcriptomics of beneficial fungi?

The different ways to communicate with their hosts and to establish compatibility in divergent ECM, AM and root endophytic fungal lineages, reflected in the different amount and expression patterns of genes encoding for example, SSPs, hydrolytic enzymes, lectins and genes involved in signal transduction, suggest that similar functional properties and outputs of interactions (e.g. phosphate transfer, growth promotion and establishment of biotrophy) have evolved independently through convergent evolution. Comparative genomic and transcriptomic data, combined with a careful analysis of the individual fungal behaviors on diverse hosts, are a valuable tool to infer lifestyle complexity, aiding in the identification of the symbiosis determinants and their evolution.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Bonfante P, Genre A: **Mechanisms underlying beneficial plant–fungus interactions in mycorrhizal symbiosis**. *Nat Commun* 2010, **1**:48.

2. Nehls U, Bodendiek I: **Carbohydrates exchange between symbionts in ectomycorrhizas**. In *Fungal associations, The Mycota IX 2nd Edition*, vol 9. Edited by Hock B. Berlin Heidelberg: Springer-Verlag; 2012:119-136.

3. Gutjahr C, Parniske M: **Cell and developmental biology of arbuscular mycorrhiza symbiosis**. *Annu Rev Cell Dev Biol* 2013, **29**:593-617.

4. Lahrmann U, Zuccaro A: **Opprimo ergo sum—evasion and suppression in the root endophytic fungus *Piriformospora indica***. *Mol Plant Microbe Interact* 2012, **25**:727-737.

5. Harrison MJ: **Cellular programs for arbuscular mycorrhizal symbiosis**. *Curr Opin Plant Biol* 2012, **15**:691-698.

6. Smith SE, Read DJ: *Mycorrhizal symbiosis*. 3rd ed.. Academic Press; 2008.

7. •• Tisserant E, Malbreil M, Kuo A, Kohler A, Symeonidi A, Balestrini R, Charron P, Duensing N, Frei dit Frey N, Gianinazzi-Pearson V *et al.*: **Genome of an arbuscular mycorrhizal fungus provides insight into the oldest plant symbiosis**. *Proc Natl Acad Sci U S. A* 2013, **110**:20117-20122.

This paper provides genome and transcriptome analyses of the arbuscular endomycorrhizal fungus *Rhizophagus irregularis*. The data reveal that obligate biotrophy in AM fungi is not explained by genome erosion or loss of metabolic complexity in central metabolism, but is marked by the lack of genes involved in plant cell wall degradation, toxin-, and thiamine-synthesis.

8. •• Lin K, Limpens E, Zhang Z, Ivanov S, Saunders DG, Mu D, Pang E, Cao H, Cha H, Lin T *et al.*: **Single nucleus genome sequencing reveals high similarity among nuclei of an endomycorrhizal fungus**. *PLoS Genet* 2014, **10**:e1004078.

This paper provides hints into the genetic makeup of the arbuscular endomycorrhizal fungus *Rhizophagus irregularis* by sequencing four individual nuclei. The data reveal a surprisingly low level of polymorphism between nuclei indicating that this fungal stain is homokaryotic.

9. Plett JM, Martin F: **Blurred boundaries: lifestyle lessons from ectomycorrhizal fungal genomes**. *Trends Genet* 2011, **27**:14-22.

10. Hibbett DS, Matheny PB: **The relative ages of ectomycorrhizal mushrooms and their plant hosts estimated using Bayesian relaxed molecular clock analyses**. *BMC Biol* 2009, **7**:13.

11. •• Martin F, Aerts A, Ahren D, Brun A, Danchin EG, Duchaussoy F, Gibon J, Kohler A, Lindquist E, Pereda V *et al.*: **The genome of *Laccaria bicolor* provides insights into mycorrhizal symbiosis**. *Nature* 2008, **452**:88-92.

This paper describes the first genome of a mutualistic ectomycorrhizal basidiomycete fungus, opening the door to a deeper understanding of the processes by which symbionts interacts with plants.

12. •• Martin F, Kohler A, Murat C, Balestrini R, Coutinho PM, Jaillon O, Montanini B, Morin E, Noel B, Percudani R *et al.*: **Perigord black truffle genome uncovers evolutionary origins and mechanisms of symbiosis**. *Nature* 2010, **464**:1033-1038.

This paper describes the sequence analysis of the haploid genome and transcriptome of the ectomycorrhizal ascomycete fungus *Tuber melanosporum*. Despite a large genome with 125 Mbp, only 7500 protein-coding genes with very rare multigene families were found. In planta expression analysis suggests that this symbiont degrades host cell walls during colonization.

13. Johnson NC, Wilson GWT, Bowker MA, Wilson JA, Miller RM: **Resource limitation is a driver of local adaptation in mycorrhizal symbioses**. *Proc Natl Acad Sci U S A* 2010, **107**:2093-2098.

14. Plattner I, Hall IR: **Parasitism of nonhost plants by the mycorrhizal fungus *Tuber melanosporum***. *Mycol Res* 1995, **99**:1367-1370.

15. Jones MD, Smith SE: **Exploring functional definitions of mycorrhizas: Are mycorrhizas always mutualisms?** *Can J Bot* 2004, **10**:9-1109.

16. Hoeksema JD: **Ongoing coevolution in mycorrhizal interactions**. *New Phytol* 2010, **187**:286-300.

17. Schulz B, Boyle C: **The endophytic continuum**. *Mycol Res* 2005, **109**:661-686.

18. Brundrett M: **Diversity and classification of mycorrhizal associations**. *Biol Rev Camb Philos Soc* 2004, **79**:473-495.

19. Jacobs S, Zechmann B, Molitor A, Trujillo M, Petutschnig E, Lipka V, Kogel KH, Schäfer P: **Broad-spectrum suppression of innate immunity is required for colonization of Arabidopsis**

(Figure 4 Legend Continued) The numbers of predicted functional protein domains are represented as $\log_2$-transformed values in the heatmap. Hierarchical clustering was applied based on euclidean distances and Ward's minimum variance method using the hclust function of the R package, version 3.0.2 [74]. The uncertainty in the clustering was assessed using the pvclust package [75]. Numbers given in the column-dendogram refer to AU (Approximately Unbiased) *p*-values calculated by pvclust which are determined by multiscale bootstrap resampling. Species labels on the *x*-axis contain the following additional information for classification: One-letter codes describing the division: A — Ascomycota; B — Basidiomycota; G — Glomeromycota. Three-letter codes describing the order: Glo — Glomerales; Puc — Pucciniales; Ery — Erysiphales; Pez — Pezizales; Mix — Mixiales; Ust — Ustilaginales; Tre — Tremellales; Mal — Malasseziales; Hyp — Hypocreales; Hel — Helotiales; Cap — Capnodiales; Gll — Glomerellales; Mag — Magnaporthales; Aga — Agaricales; Seb — Sebacinales; Aur — Auriculariales; Pol — Polyporales; Cor — Corticiales; Rus — Russulales;

roots by the fungus *Piriformospora indica*. *Plant Physiol* 2011, **156**:726-740.

20. • Lahrmann U, Ding Y, Banhara A, Rath M, Hajirezaei MR, Döhlemann S, von Wirén N, Parniske M, Zuccaro A: **Host-related metabolic cues affect colonization strategies of a root endophyte**. *Proc Natl Acad Sci U S. A* 2013, **110**:13965-13970.
This paper describes the colonization strategies of the root endophyte *Piriformospora indica* at different symbiotic stages in the monocot barley and in the dicot *Arabidopsis thaliana*, via comparative gene expression and cytological analyses. This work allowed the identification of a common set of genes induced in planta and a gene set specifically expressed depending on the host.

21. Behie SW, Zelisko PM, Bidochka MJ: **Endophytic insect-parasitic fungi translocate nitrogen directly from insects to plants**. *Science* 2012, **336**:1576-1577.

22. Yadav V, Kumar M, Deep DK, Kumar H, Sharma R, Tripathi T, Tuteja N, Saxena AK, Johri AK: **A phosphate transporter from the root endophytic fungus *Piriformospora indica* plays a role in phosphate transport to the host plant**. *J Biol Chem* 2010, **285**:26532-26544.

23. Usuki F, Narisawa K: **A mutualistic symbiosis between a dark septate endophytic fungus, *Heteroconium chaetospira*, and a nonmycorrhizal plant, Chinese cabbage**. *Mycologia* 2007, **99**:175-184.

24. Weiss M, Selosse MA, Rexer KH, Urban A, Oberwinkler F: **Sebacinales: a hitherto overlooked cosm of heterobasidiomycetes with a broad mycorrhizal potential**. *Mycol Res* 2004, **108**:1003-1010.

25. • Kubicek CP, Herrera-Estrella A, Seidl-Seiboth V, Martinez DA, Druzhinina IS, Thon M, Zeilinger S, Casas-Flores S, Horwitz BA, Mukherjee PK *et al.*: **Comparative genome sequence analysis underscores mycoparasitism as the ancestral life style of Trichoderma**. *Genome Biol* 2011, **12**:R40.
In this paper the genome sequences of two mycoparasitic species of Trichoderma were analyzed and compared to the genome sequence of a saprotrophic Trichoderma species. Several gene families were expanded in the genomes of the mycoparasitic species in non-syntenic regions. The authors suggest that mycoparasitism-specific genes may arose in a common ancestor but were lost in the saprotrophic species.

26. Mukherjee PK, Horwitz BA, Herrera-Estrella A, Schmoll M, Kenerley CM: **Trichoderma research in the genome era**. *Annu Rev Phytopathol* 2013, **51**:105-129.

27. Weiss M, Sykorova Z, Garnica S, Riess K, Martos F, Krause C, Oberwinkler F, Bauer R, Redecker D: **Sebacinales everywhere: previously overlooked ubiquitous fungal endophytes**. *PLoS ONE* 2011, **6**:e16793.

28. Veiga RS, Faccio A, Genre A, Pieterse CM, Bonfante P, van der Heijden MG: **Arbuscular mycorrhizal fungi reduce growth and infect roots of the non-host plant *Arabidopsis thaliana***. *Plant Cell Environ* 2013, **36**:1926-1937.

29. •• Zuccaro A, Lahrmann U, Guldener U, Langen G, Pfiffi S, Biedenkopf D, Wong P, Samans B, Grimm C, Basiewicz M *et al.*: **Endophytic life strategies decoded by genome and transcriptome analyses of the mutualistic root symbiont *Piriformospora indica***. *PLoS Pathog* 2011, **7**:e1002290.
This paper provides a global characterization of fungal transcriptional responses associated with the colonization of living and dead barley roots allowing the identification of a gene set characteristic for saprotrophy and a gene set involved in the establishment of biotrophy. The data suggest a series of incremental shifts along the continuum from saprotrophy towards biotrophy in the evolution of root associations from decomposer fungi.

30. Bonfante P, Requena N: **Dating in the dark: how roots respond to fungal signals to establish arbuscular mycorrhizal symbiosis**. *Curr Opin Plant Biol* 2011, **14**:451-457.

31. Xie XN, Yoneyama K, Yoneyama K: **The strigolactone story**. *Annu Rev Phytopathol* 2010, **48**:93-117.

32. Genre A, Chabaud M, Timmers T, Bonfante P, Barker DG: **Arbuscular mycorrhizal fungi elicit a novel intracellular apparatus in *Medicago truncatula* root epidermal cells before infection**. *Plant Cell* 2005, **17**:3489-3499.

33. Hückelhoven R, Panstruga R: **Cell biology of the plant-powdery mildew interaction**. *Curr Opin Plant Biol* 2011, **14**:738-746.

34. Kistner C, Winzer T, Pitzschke A, Mulder L, Sato S, Kaneko T, Tabata S, Sandal N, Stougaard J, Webb KJ *et al.*: **Seven *Lotus japonicus* genes required for transcriptional reprogramming of the root during fungal and bacterial symbiosis**. *Plant Cell* 2005, **17**:2217-2229.

35. Singh S, Katzer K, Lambert J, Cerri M, Parniske M: **CYCLOPS, a DNA-binding transcriptional activator, orchestrates symbiotic root nodule development**. *Cell Host Microbe* 2014, **15**:139-152.

36. Delaux PM, Sejalon-Delmas N, Becard G, Ane JM: **Evolution of the plant–microbe symbiotic 'toolkit'**. *Trends Plant Sci* 2013, **18**:298-304.

37. Agerer R: **Characterization of ectomycorrhiza**. *Method Microbiol* 1991, **23**:25-73.

38. Kottke I, Oberwinkler F: **Mycorrhiza of forest trees — structure and function**. *Trees Struct Funct* 1986, **1**:1-24.

39. Martin F, Nehls U: **Harnessing ectomycorrhizal genomics for ecological insights**. *Curr Opin Plant Biol* 2009, **12**:508-515.

40. Desai S, Naik D, Cumming JR: **The influence of phosphorus availability and *Laccaria bicolor* symbiosis on phosphate acquisition, antioxidant enzyme activity, and rhizospheric carbon flux in *Populus tremuloides***. *Mycorrhiza* 2013 http://dx.doi.org/10.07/s00572-013-0548-1.

41. Deshmukh S, Hückelhoven R, Schäfer P, Imani J, Sharma M, Weiss M, Waller F, Kogel KH: **The root endophytic fungus *Piriformospora indica* requires host cell death for proliferation during mutualistic symbiosis with barley**. *Proc Natl Acad Sci U S A* 2006, **103**:18450-18457.

42. Ye W, Shen CH, Lin Y, Chen PJ, Xu X, Oelmüller R, Yeh KW, Lai Z: **Growth promotion-related miRNAs in *Oncidium* orchid roots colonized by the endophytic fungus *Piriformospora indica***. *PLoS ONE* 2014, **9**:e84920.

43. Dong S, Tian Z, Chen PJ, Senthil Kumar R, Shen CH, Cai D, Oelmüller R, Yeh KW: **The maturation zone is an important target of *Piriformospora indica* in Chinese cabbage roots**. *J Exp Bot* 2013, **64**:4529-4540.

44. Peskan-Berghofer T, Shahollari B, Giong PH, Hehl S, Markert C, Blanke V, Kost G, Varma A, Oelmüller R: **Association of *Piriformospora indica* with *Arabidopsis thaliana* roots represents a novel system to study beneficial plant–microbe interactions and involves early plant protein modifications in the endoplasmic reticulum and at the plasma membrane**. *Physiol Plant* 2004, **122**:465-477.

45. Karandashov V, Nagy R, Wegmuller S, Amrhein N, Bucher M: **Evolutionary conservation of a phosphate transporter in the arbuscular mycorrhizal symbiosis**. *Proc Natl Acad Sci U S A* 2004, **101**:6285-6290.

46. Qiang X, Zechmann B, Reitz MU, Kogel KH, Schäfer P: **The mutualistic fungus *Piriformospora indica* colonizes Arabidopsis roots by inducing an endoplasmic reticulum stress-triggered caspase-dependent cell death**. *Plant Cell* 2012, **24**:794-809.

47. Spanu PD: **The genomics of obligate (and nonobligate) biotrophs**. *Annu Rev Phytopathol* 2012, **50**:91-109.

48. Horst RJ, Döhlemann G, Wahl R, Hofmann J, Schmiedl A, Kahmann R, Kämper J, Sonnewald U, Voll LM: ***Ustilago maydis* infection strongly alters organic nitrogen allocation in maize and stimulates productivity of systemic source leaves**. *Plant Physiol* 2010, **152**:293-308.

49. Tschaplinski TJ, Plett JM, Engle N, Deveau A, Cushman K, Martin MZ, Doktycz MJ, Tuskan G, Brun A, Kohler A *et al.*: ***Populus trichocarpa* and *Populus deltoides* exhibit different metabolomic responses to colonization by the symbiotic fungus *Laccaria bicolor***. *Mol Plant Microbe Interact* 2014 http://dx.doi.org/10.1094/MPMI-09-13-0286-R.

50. Bonfante P, Genre A: **Plants and arbuscular mycorrhizal fungi: an evolutionary-developmental perspective**. *Trends Plant Sci* 2008, **13**:492-498.

51. Hetrick BAD, Bloom J, Feyerherm SM: **Root colonization pattern of *Glomus epigaeum* in nine host species**. *Mycologia* 1985, **77**:825-828.

52. Boyetchko SM, Tewari JP: **Root colonization of different hosts by the vesicular–arbuscular mycorrhizal fungus *Glomus dimorphicum***. *Plant Soil* 1990, **129**:131-136.

53. • Spanu PD, Abbott JC, Amselem J, Burgis TA, Soanes DM, Stuber K, Ver Loren van Themaat E, Brown JK, Butcher SA, Gurr SJ *et al.*: **Genome expansion and gene loss in powdery mildew fungi reveal tradeoffs in extreme parasitism**. *Science* 2010, **330**:1543-1546.
In this paper genome sequence analyses of barley powdery mildew fungus, as well as a comparison with the analyses of two powdery mildew fungi pathogenic on dicot plants were performed. The genomes display a strong size-expansion and gene losses in primary and secondary metabolism, carbohydrate-active enzymes and transporters, suggesting adaptation to an exclusively biotrophic lifestyle.

54. • Duplessis S, Cuomo CA, Lin YC, Aerts A, Tisserant E, Veneault-Fourrey C, Joly DL, Hacquard S, Amselem J, Cantarel BL *et al.*: **Obligate biotrophy features unraveled by the genomic analysis of rust fungi**. *Proc Natl Acad Sci U S. A* 2011, **108**:9166-9171.
Data presented in this paper identified genomic features related to obligate biotrophy, including expanded lineage-specific gene families, a large repertoire of effector candidates, impaired nitrogen and sulfur assimilation and expansion for amino-acids and oligopeptide membrane transporters.

55. Stogios PJ, Prive GG: **The BACK domain in BTB-kelch proteins**. *Trends Biochem Sci* 2004, **29**:634-637.

56. Lanfranco L, Young JPW: **Genetic and genomic glimpses of the elusive arbuscular mycorrhizal fungi**. *Curr Opin Plant Biol* 2012, **15**:454-461.

57. Veneault-Fourrey C, Martin F: **Mutualistic interactions on a knife-edge between saprotrophy and pathogenesis**. *Curr Opin Plant Biol* 2011, **14**:444-450.

58. • O'Connell RJ, Thon MR, Hacquard S, Amyotte SG, Kleemann J, Torres MF, Damm U, Buiate EA, Epstein L, Alkan N *et al.*: **Lifestyle transitions in plant pathogenic *Colletotrichum* fungi deciphered by genome and transcriptome analyses**. *Nat Genet* 2012, **44**:1060-1065.
This paper provides genome and transcriptome analyses of the hemibiotrophic fungi *Colletotrichum higginsianum* infecting *Arabidopsis thaliana* and *C. graminicola* infecting maize. Data suggest that these two fungi use very different strategies to deconstruct plant cell walls reflecting their host preferences.

59. Akiyama K, Matsuzaki K, Hayashi H: **Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi**. *Nature* 2005, **435**:824-827.

60. Genre A, Chabaud M, Balzergue C, Puech-Pages V, Novero M, Rey T, Fournier J, Rochange S, Becard G, Bonfante P *et al.*: **Short-chain chitin oligomers from arbuscular mycorrhizal fungi trigger nuclear Ca^{2+} spiking in *Medicago truncatula* roots and their production is enhanced by strigolactone**. *New Phytol* 2013, **198**:190-202.

61. Maillet F, Poinsot V, Andre O, Puech-Pages V, Haouy A, Gueunier M, Cromer L, Giraudet D, Formey D, Niebel A *et al.*: **Fungal lipochitooligosaccharide symbiotic signals in arbuscular mycorrhiza**. *Nature* 2011, **469**:58-63.

62. Kloppholz S, Kuhn H, Requena N: **A secreted fungal effector of *Glomus intraradices* promotes symbiotic biotrophy**. *Curr Biol* 2011, **21**:1204-1209.

63. Pumplin N, Zhang X, Noar RD, Harrison MJ: **Polar localization of a symbiosis-specific phosphate transporter is mediated by a transient reorientation of secretion**. *Proc Natl Acad Sci U S A* 2012, **109**:E665-E672.

64. Felten J, Kohler A, Morin E, Bhalerao RP, Palme K, Martin F, Ditengou FA, Legue V: **The ectomycorrhizal fungus *Laccaria bicolor* stimulates lateral root formation in poplar and Arabidopsis through auxin transport and signaling**. *Plant Physiol* 2009, **151**:1991-2005.

65. Plett JM, Kemppainen M, Kale SD, Kohler A, Legue V, Brun A, Tyler BM, Pardo AG, Martin F: **A secreted effector protein of *Laccaria bicolor* is required for symbiosis development**. *Curr Biol* 2011, **21**:1197-1203.

66. Plett JM, Khachane A, Ouassou M, Sundberg B, Kohler A, Martin F: **Ethylene and jasmonic acid act as negative modulators during mutualistic symbiosis between *Laccaria bicolor* and Populus roots**. *New Phytol* 2014, **202**:270-286.

67. Hilbert M, Voll LM, Ding Y, Hofmann J, Sharma M, Zuccaro A: **Indole derivative production by the root endophyte *Piriformospora indica* is not required for growth promotion but for biotrophic colonization of barley roots**. *New Phytol* 2012, **196**:520-534.

68. Hilbert M, Nostadt R, Zuccaro A: **Exogenous auxin affects the oxidative burst in barley roots colonized by *Piriformospora indica***. *Plant Signal Behav* 2013, **8**:e23572.

69. Schäfer P, Pfiffi S, Voll LM, Zajic D, Chandler PM, Waller F, Scholz U, Pons-Kühnemann J, Sonnewald S, Sonnewald U *et al.*: **Manipulation of plant innate immunity and gibberellin as factor of compatibility in the mutualistic association of barley roots with *Piriformospora indica***. *Plant J* 2009, **59**:461-474.

70. Finn RD, Bateman A, Clements J, Coggill P, Eberhardt RY, Eddy SR, Heger A, Hetherington K, Holm L, Mistry J *et al.*: **Pfam: the protein families database**. *Nucleic Acids Res* 2014, **42**: D222-D230.

71. • de Wit PJ, van der Burgt A, Okmen B, Stergiopoulos I, Abd-Elsalam KA, Aerts AL, Bahkali AH, Beenen HG, Chettri P, Cox MP *et al.*: **The genomes of the fungal plant pathogens *Cladosporium fulvum* and *Dothistroma septosporum* reveal adaptation to different hosts and lifestyles but also signatures of common ancestry**. *PLoS Genet* 2012, **8**:e1003088.
In this paper comparative genomics of the biotrophic fungus *Cladosporium fulvum* and the hemibiotrophic fungus *Dothistroma septosporum* identified recent adaptation to different hosts and lifestyles by a combination of diverged sets of genes (e.g. an alfa-tomatinase), pseudogenization, and gene regulation (e.g. carbohydrate-degrading enzymes).

72. Blin K, Medema MH, Kazempour D, Fischbach MA, Breitling R, Takano E, Weber T: **antiSMASH 2.0—a versatile platform for genome mining of secondary metabolite producers**. *Nucleic Acids Res* 2013, **41**:W204-W212.

73. Grigoriev IV, Nikitin R, Haridas S, Kuo A, Ohm R, Otillar R, Riley R, Salamov A, Zhao X, Korzeniewski F *et al.*: **MycoCosm portal: gearing up for 1000 fungal genomes**. *Nucleic Acids Res* 2014, **42**:D699-D704.

74. R-Core-Team: *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing; 2012.

75. Suzuki R, Shimodaira H: **pvclust: an R package for assessing the uncertainty in hierarchical clustering**. *Bioinformatics* 2006, **22**:1540-1542.